Chapter 23
The Metabolism of Nitrogen

SUMMARY

Section 23.1

- Atmospheric nitrogen (N_2) is not highly reactive, but it must be converted to ammonia or to nitrates to be biologically useful, first to plants, then to animals.
- Nitrogen compounds in the biosphere include amino acids, purines and pyrimidines. Their pathways for biosynthesis and breakdown tend to be long and complex.

Section 23.2

- Nitrogen enters the biosphere by the process of nitrogen fixation. Atmospheric nitrogen is converted to ammonia in its conjugate acid form, ammonium ion.
- The nitrogenase enzyme found in root nodules of leguminous plants catalyzes crucial reactions in nitrogen fixation.

Section 23.3

- Because the biosynthetic pathways for many nitrogen-containing compounds are long and complex, organisms save energy by inactivating these pathways when the compounds in question are not needed. This is frequently achieved by feedback inhibition.

Section 23.4

- Two of the most important classes of reactions in the biosynthesis of amino acids are transamination reactions and one-carbon transfers.
- The amino acids glutamate and glutamine are the principal donors of amino groups in transamination reactions.
- Carriers of one-carbon groups include biotin, *S*-adenosylmethionine, and derivatives of folic acid.

Section 23.5

- Humans cannot produce some amino acids in sufficient quantities to meet their metabolic needs. They are called essential amino acids.
- The essential amino acids must be obtained from dietary sources.

Section 23.6

- The carbon skeleton has two fates in the breakdown process. Some carbon skeletons give rise to pyruvate or oxaloacetate, which can be used in gluconeogenesis. Others give rise to acetyl-CoA or acetoacetyl-CoA, which can form lipids.
- The urea cycle, which has links to the citric acid cycle, plays a central role in nitrogen metabolism. It is involved in both the anabolism and the catabolism of amino acids.

Section 23.7

- The growing ring system of purines is attached to ribose phosphate during the synthesis process.
- The biosynthesis of nucleotides requires considerable expenditures of energy by organisms in long and complex pathways. Feedback inhibition at all stages plays a key role in regulating the pathway.

Section 23.8

- Purines are degraded to uric acid in primates (including humans) and further degraded in other organisms. Overproduction of uric acid causes gout in humans.
- Salvage reactions exist for reuse of some of purines.

Section 23.9

- The ring system of pyrimidines is assembled before it is attached to ribose phosphate.
- During breakdown, the nucleoside is formed first, then the base. Ring opening reactions of the base complete the degradation.

Section 23.10

- NADPH serves as the ultimate reducing agent in a process that requires several enzymes and intermediate electron carriers.

Section 23.11

- The addition of a methyl group to uracil to produce thymine requires tetrahydrofolate as the one-carbon carrier. This process is a target for cancer chemotherapy.

LECTURE NOTES

This chapter covers both the anabolism and catabolism of nitrogen-containing compounds. Students generally find this material interesting because of the clinical implications of inborn errors of metabolism involving these compounds and because of readily apparent relationships to everyday nutrition. The material can be overwhelming however, as there is not one over-riding linear pathway to follow, such as glycolysis. This chapter may several lectures to complete: The basics of nitrogen incorporation into the biosphere could be one lecture in itself. Amino acid metabolism may take one to two lectures. Nucleotide metabolism is also likely to require at least two lectures.

LECTURE OUTLINE

I. Overview of nitrogen metabolism
    A. Fixation
    B. Nitrification
    C. Denitrification
    D. Metabolism of amino acids, purines, pyrimidines, and porphyrins
II. Nitrogen Fixation
    A. Reduction of N2 to ammonia

- B. Nitrogenase
  1. Eight electrons
  2. 16 ATP
  3. Ferredoxin
  4. Dinitrogenase reductase
  5. Use of molybdenum

III. Role of feedback inhibition
IV. Amino acid biosynthesis
- A. General features
- B. Transamination reactions
  1. Glutamate dehydrogenase
  2. Glutamine synthetase
  3. Pyridoxal phosphate
- C. One-carbon transfers and the serine family
  1. Tetrahydrofolate
  2. S-adenosylmethionine

V. Essential amino acids
VI. Amino acid catabolism
- A. Disposition of carbon skeletons
  1. Glucogenic amino acids
  2. Ketogenic amino acids
- B. Excretion of excess nitrogen
- C. Urea cycle
  1. Carbamoyl phosphate
  2. Ornithine
  3. Citrulline
  4. Argininosuccinate
  5. Arginine and fumarate
  6. Hydrolysis yielding urea and regenerating ornithine
  7. Link with citric acid cycle

VII. Purine biosynthesis
- A. Anabolism of inosine monophosphate
- B. Conversion of IMP to AMP and GMP
- C. Energy requirements

VIII. Purine catabolism
IX. Pyrimidine metabolism
- A. Anabolism of pyrimidine nucleotides
- B. Pyrimidine catabolism

X. Deoxyribonucleotide synthesis
XI. Conversion of dUDP to dTTP

ANSWERS TO PROBLEMS

23.1 Nitrogen Metabolism: An Overview

1. Nitrogen-fixing bacteria (symbiotic organisms that form nodules on the roots of leguminous plants, such as beans and alfalfa) and some free-living microbes and cyanobacteria can fix nitrogen. Plants and animals cannot.

23.2 Nitrogen Fixation

2. Nitrogen is fixed by the nitrogenase reaction, in which N_2 is converted to NH_4^+. Very few organisms have this enzyme, which can catalyze the breaking of the triple bond in molecular nitrogen. The glutamate dehydrogenase reaction and the glutamine synthase reactions assimilate nitrogen:

$$NH_4^+ + \alpha\text{-Ketoglutarate} \rightleftharpoons \text{Glutamate} + \text{Water (NADPH required)}$$

$$NH_4^+ + \text{Glutamate} \rightleftharpoons \text{Glutamine (ATP required)}$$

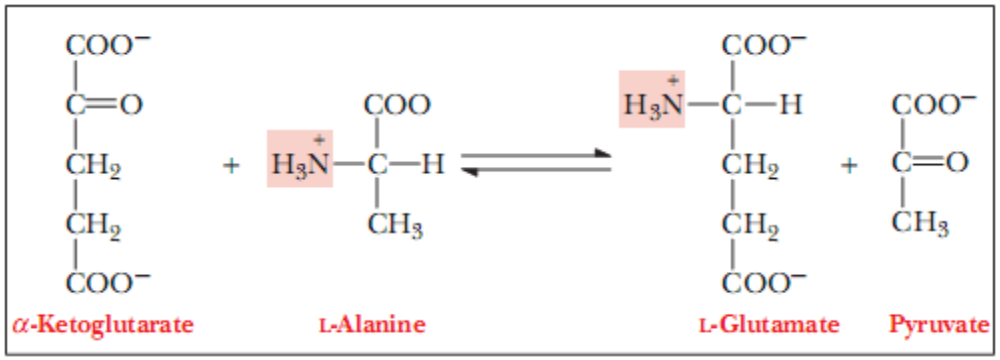
3. The chemical synthesis of ammonia from H_2 and N_2.
4. $N_2 + 8e^- + 16ATP + 10H^+ \rightarrow 2NH_4^+ + 16ADP + 16P_i + H_2$ is the half reaction for reduction via nitrogenase. The oxidation reaction varies with species.
5. The nitrogenase complex is made up of ferredoxin, dinitrogenase reductase, and nitrogenase. Dinitrogenase reductase is an iron–sulfur protein, whereas nitrogenase is an iron–molybdenum protein. The Fe—S protein is a dimer ("the iron butterfly"), with the iron–sulfur cluster located at the butterfly's head. The nitrogenase is even more complicated, with several types of subunits arranged into tetramers.

23.3 Feedback Inhibition in Nitrogen Metabolism

6. Pathways that use nitrogen to make amino acids, purines, and pyrimidines are controlled by feedback inhibition. The final product, such as CTP, inhibits the first or an early step in its synthesis.
7. Feedback control mechanisms slow down long biosynthetic pathways at or near their beginnings, saving energy for the organism.
8. Because all the components of a cycle are regenerated, only small amounts ("catalytic quantities") are needed. This is important from an energy standpoint and, perhaps with some compounds, because of insolubility problems.

23.4 Amino Acid Biosynthesis

9. They are all interrelated. α-Ketoglutarate can be changed to glutamate via transamination or glutamate dehydrogenase. Glutamine synthetase makes glutamine out of glutamate.
10.



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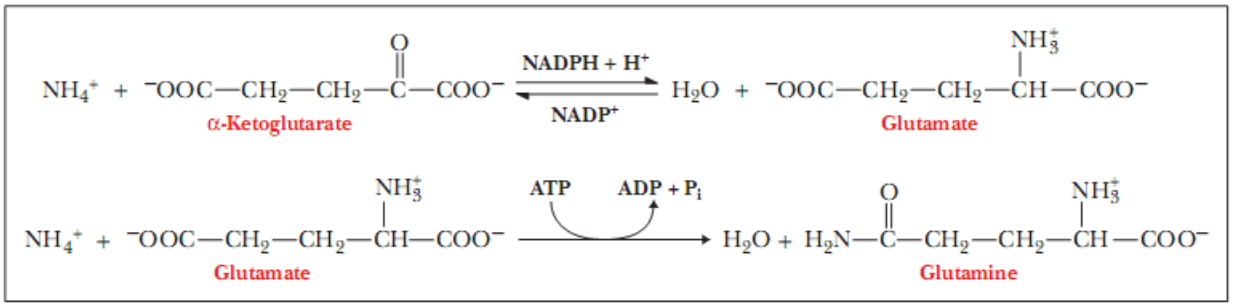

12. Glutamine synthetase catalyzes the following reaction and uses energy: NH_4^+ + Glutamate + ATP → Glutamine + ADP + P_i + H_2O. Glutaminase catalyzes the following reaction and does not use energy directly: Glutamine + H_2O → Glutamate + NH_4^+.
13. See Figure 23.8.
14. The principal ones are tetrahydrofolate and *S*-adenosylmethionine.
15. See Figure 23.11.
16. Conversion of homocysteine to methionine using *S*-adenosylmethionine as the methyl donor gives no net gain; one methionine is needed to produce another methionine.
17. Glutamate + α-Keto acid → α-Ketoglutarate + Amino acid
18. See the *S*-adenosylmethionine structure in Figure 23.15. The reactive methyl group is indicated.
19. Sulfanilamide inhibits folic acid biosynthesis.
20. Methionine can play a dual role. In addition to providing a hydrophobic group, methionine (in the form of *S*-adenosylmethionine) can act as a methyl group donor.

23.5 Essential Amino Acids

21. The essential amino acids are those with branched chains, aromatic rings, or basic side chains.
22. In both cases, the requirements are those given in Table 23.1.

23.6 Amino Acid Catabolism

23. Five α-amino acids are involved directly in the urea cycle (ornithine, citrulline, aspartate, arginosuccinate, and arginine). Of those, only aspartate and arginine are also found in proteins.
24. $H^+ + HCO_3^- + 2NH_3 + 3ATP \rightarrow NH_2CONH_2 + 2ADP + 2P_i + AMP + PP_i + 2H_2O$. The urea cycle is linked to the citric acid cycle by fumarate and by aspartate, which can be converted to malate by transamination (see Figure 23.19).
25. Ornithine is similar to lysine, but it has one fewer methylene group in the side chain. Citrulline is a keto version of arginine with a side chain $C{=}NH_2^+$ replaced by C=O.
26. Aspartate and arginosuccinate are the amino acids that link the two pathways. Aspartate is made by transamination of OAA. The aspartate then combines with citrulline to form arginosuccinate, which then releases a fumarate to go back to the TCA cycle.

27. Each round of the urea cycle costs 4 ATP, two to make carbamoyl-phosphate and effectively two (ATP ~~3~~→ AMP) to make arginosuccinate.
28. It is controlled by a special effector molecule, *N*-acetylglutamate, which is itself controlled by levels of arginine.
29. When arginine levels build up, it means that the urea cycle is going too slow and not enough carbamoyl-phosphate is available to react with ornithine.
30. Glutamate brings ammonia groups to the matrix of the mitochondria for the urea cycle. High levels of glutamate stimulate the urea cycle.
31. Glucogenic amino acids are degraded to pyruvate or one of the citric acid cycle intermediates found after the decarboxylation steps, such as succinate or malate. Ketogenic amino acids are degraded to acetyl-CoA or acetoacetyl-CoA.
32.

(a) Glucogenic
(b) Glucogenic
(c) Glucogenic
(d) Ketogenic
(e) Glucogenic
(f) Ketogenic

33. Fish excrete excess nitrogen as ammonia, and birds excrete it as uric acid. Mammals excrete it as urea.
34. Because ostriches don't fly, one could argue that they would excrete their excess nitrogen as urea. On the other hand, they are birds, and as such probably have the same metabolism of their lighter counterparts, and might likely excrete it as uric acid.
35. The amounts of arginine necessary in the urea cycle are only catalytic. If arginine from the cycle is used for protein synthesis, the cycle becomes depleted.
36. A high-protein diet leads to increased production of urea. Drinking more water increases the volume of urine, ensuring elimination of the urea from the body with less strain on the kidneys than if urea were at a higher concentration.
37. The metabolism of amino acids encourages urine formation and actually a greater thirst and need for water.
38. Several enzymes, resulting from mutations, are needed for the urea cycle. Most mutations tend to be lost unless they provide some survival value. It seems improbable that all the mutations needed for all the enzymes of the cycle would arise nearly simultaneously. However, the origin of the cycle can be rather easily explained on the premise that only one new enzyme (arginase) was needed. The other enzymes of the cycle are needed for the biosynthesis of arginine. As a component of proteins, arginine was presumably needed before there was a need for a urea cycle. This is an example of nature using features already available to bring about a new function.

23.7 Purine Biosynthesis

39. Since folic acid is critical to the formation of purines, antagonists of folic acid metabolism are used as chemotherapy drugs to inhibit nucleic acid synthesis and cell growth. Rapidly dividing cells, such as those found in cancer and tumors, are more susceptible to these antagonists.

40. All four nitrogen atoms of the purine ring are derived from amino acids: two from glutamine, one from aspartate, and one from glycine. Two of the five carbon atoms (adjacent to the glycine nitrogen) also come from glycine, two more come from tetrahydrofolate derivatives, and the fifth comes from CO_2.
41. In inosine, carbon-6 of the ring is a ketone group; in adenosine, carbon-6 is bound to an amino group.
42. Tetrahydrofolate is a carrier of carbon groups. Two of the carbons in the purine ring are donated by tetrahydrofolate.
43. The conversion of IMP to GMP produces one NADH and uses the equivalent of 2 ATP because an ATP is converted to AMP. Because NADH gives rise to 2.5 ATP if it goes into the electron transport chain, we can say that the conversion results in a net production of ATP.
44. There is a complicated system of feedback inhibition for the production of purine-containing nucleotides. The final products, ATP and GTP, feed back to inhibit the first steps starting from ribose-5-phosphate. In addition, each intermediate, such as AMP or ADP, can also inhibit the first step. Also, each of the three forms for each nucleotide inhibit the committed reaction from IMP that eventually decides which purine nucleotide is made.

23.8 Purine Catabolism

45. The purine salvage reaction that produces GMP requires the equivalent of 2 ATP. The pathway to IMP and then to GMP requires the equivalent of 8 ATP.
46. In most mammals, uric acid is converted to allantoic acid, which is much more water soluble than uric acid.

23.9 Pyrimidine Biosynthesis and Catabolism

47. In purine nucleotide biosynthesis, the growing purine ring is covalently bonded to ribose; the ribose is added after the ring is synthesized in pyrimidine nucleotide biosynthesis.
48. Purines break down to various products, depending on the species. These products are then excreted, representing a major means of nitrogen excretion for many organisms. Pyrimidine catabolism yields, in addition to NH_4^+ and CO_2, the salvageable product β-alanine, which is a breakdown product of both cytosine and uracil.

23.10 Conversion of Ribonucleotides to Deoxyribonucleotides

49. Both thioredoxin and thioredoxin reductase are proteins involved in the conversion of ribonucleotides to deoxyribonucleotides. Thioredoxin is an intermediate carrier of electrons and hydrogens, and thioredoxin reductase is the enzyme that catalyzes the process.

23.11 Conversion of dUTP to dTTP

50. Fluorouracil substitutes for thymine in DNA synthesis. In rapidly dividing cells, such as cancer cells, the result is the production of defective DNA, causing cell death.

51. The DNA of fast-growing cells, such as those of the hair follicles, is damaged by chemotherapeutic agents.